(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 469 515 A1**

# (12) EUROPEAN PATENT APPLICATION

(21) Application number: **91112710.8**

(51) Int. Cl.5: **A61J 1/00**

(22) Date of filing: **29.07.91**

(30) Priority: **02.08.90 JP 82296/90**
**13.06.91 JP 168670/91**

(43) Date of publication of application:
**05.02.92 Bulletin 92/06**

(84) Designated Contracting States:
**DE FR GB**

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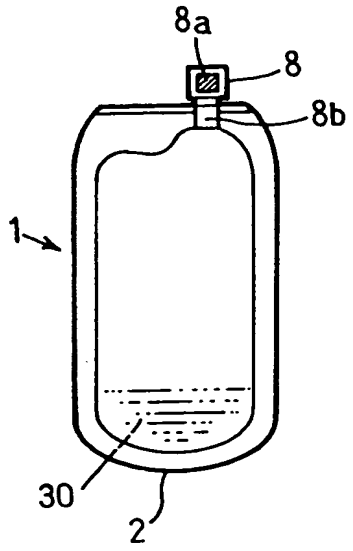
## (54) Container for preserving blood for testing.

(57) Provided is a bag-like main body container (2) made of a flexible synthetic resin and a sampling port part (8) for collecting blood components inside the main body container. The above main body container has a volume of 20 to 80 ml, and the flexible synthetic resin has oxygen permeability of not less than 0.1 ml/ 24 hr/atm per 1 ml of whole blood. Even when blood lymphocytes are preserved at room temperature for not less than 3 days, not less than 90% of the lymphocyte activity is retained.

FIG_1




EP 0 469 515 A1

## Background of the Invention

### Field of the Invention

The invention relates to a container to preserve blood for testing which enables to achieve various blood tests at high accuracy even after preserving collected blood for a long time.

### Description of the Prior Art

In organ transplantation or marrow transplantation, particularly in the latter, it is necessary to confirm that the histocompatible antigens or a donor and a recipient be compatible before transplantation. HLA, Human Leukocyte Autigen, is the major histocompatible antigen system in man.

First, after collecting lymphocytes of a donor and a recipient, the type of HLA shall be classified by having them react with various anti-HLA antisera.

Second, a compartible donor is selected by comparing HLA of the donors and the recipients.

Finally, their histocompatibility is comfirmed by examining MLC (Mixed Lymphocyte Culture) reaction.

Only 20 to 30% of the patients in need of marrow transplantation can find an HLA histocompatible related donor from their blood relatives. Other patients have to rely on the donation of marrow by a histocompatible unrelated donors. Because of this, an organization (marrow bank) which requests the cooperation of a third party in good faith, carries out typing and registering the type of HLA of many marrow donor candidates and provides histocompatible marrow to patients who need unrelated bone marrow transplantion is established.

As a conventional container to preserve blood test samples, a vacuum blood collecting tube made of glass which inside is made vacuum has been used. However, there is such a time restriction in this vacuum blood collecting tube that the above described HLA typing test has to be carried out within 24 hours after collecting blood unless a special operation such as separating lymphocytes quickly after collecting blood and freeze-preserving the lymphocytes is performed.

## Summary of the Invention

An object of the invention is to provide the best container for preserving blood which enables to preserve blood samples in good condition for testing at least after 3-days storage at room temperature. Another object of the invention is to provide a container for collected blood by aseptic operation and preserving blood components for a long periods of time keeping them aseptis.

A container for preserve blood sample according to a first embodiment of the invention is provided with a bag-like container made of flexible synthetic resin and a port part for sampling blood components inside container discribed. The above bag-like main body has a volume of 20 to 80 ml, inside which an anti-coagulant is enclosed. The above flexible synthetic resin forming the main body has oxygen permeability of not less than 0.1 ml/24 hr/atm per 1 ml of whole blood.

In a container to preserve blood samples according to a second embodiment of the invention, a first container for receiving blood components and a second container for receiving blood components are connected through a first connecting tube to the above container main body, and to the second container for receiving blood components, a container in which a diluting liquid is enclosed is connected through a second connecting tube, wherein the above first connecting tube is connected through a connecting member to the above container main body and the second container for receiving blood components, the above second connecting tube is connected through a connecting member to the above container in which a diluting liquid is enclosed, and each of the above first container for receiving blood components and the second container for receiving blood components is provided with a sampling port part for collecting blood components therein.

## Brief Description of the Drawings

Fig. 1 is a schematic drawing of the container to preserve blood for testing according to the invention.

Fig. 2 is a schematic drawing showing another example of the container to preserve blood for testing according to the invention.

Fig. 3 is a schematic drawing showing still another example of the container for preserving blood for testing according to the invention.

Fig. 4 is a graph showing the relation between days of preservation and the viability of lymphocytes when the lymphocytes are preserved using the container for preserving blood for testing according to the invention.

Fig. 5 is a graph showing the relation between the days of preservation and stimulation index when lymphocytes of blood of donor and patient are mixedly cultured using a container for preserving blood for testing according to the invention to examine the reactivity of T lymphocytes.

## Description of the Preferred Embodiments

Fig. 1 is a schematic drawing of the container to preserve blood for test according to the inven-

tion. A container 1 for preserving is provided with a sampling port part 8 in the upper end part of a bag-like main body container 2 made of flexible synthetic resin.

As flexible synthetic resin forming the above main body container 2, a material having oxygen permeability of not less than 0.10 ml/24 hr/atm per 1 ml of whole blood is used. For example, synthetic resin such as soft vinyl chloride resin, soft vinyl chloride based elastomer (for example, Esmedica, a commodity of Sekisui Kagaku), styrene based elastomer such as poly (ethylenebutylene) polystyrene block copolymer (for example, Craytone G, a commodity of Shell Chemical; Toughteck, a commodity of Asahi Kasei Kogyo), polypropylene, bridged products of ethylene-vinyl acetate copolymer (EVA), olefine based elastomer (for example, Dominos, a commodity of Nihon Polyurethane), polyethylenetelephthalate or copolymers thereof and silicone resin. The oxygen permeability is restricted within the range described above in order to keep the taking in of oxygen and carbon dioxide and the release thereof by metabolism of blood component at a level not lower than a proper level.

The main body container 2 is constructed by piling two sheets made of the above flexible synthetic resin, heat-sealing the circumference of the above sheets with the state that sampling port part 8 being inserted to the upper end part thereof and making them bag-like.

The sampling port part 8 is provided so as to be able to sample blood for testing contained in the main body container 2 through a pricking needle or inject blood for testing. As the sampling port part 8, a so-called mixed injecting part, which is used in a blood circulation circuit and the like, is used. That is, a button made of rubber is set to the widened port part of the end part of a tube 8b, and the button made of rubber is covered with a cap. This cap is provided with an opening part so that the button made of rubber can be pricked with a needle. Since there are cases that the button made of rubber is pricked with a needle a plurality of times in the invention, it is preferred that the button made of rubber is press-set to the above widened port part of the tube and to the cap using an ultrasonic welder so that a hole formed on the button by being pricked with a needle can easily be closed after pricking.

In order to improve the preservability of blood and blood components, an anti-coagulating agent 30 such as an ACD liquid (containing citric acid and glucose as main ingredients), a CPD liquid (containing citric acid and glucose as main ingredients), or a 4% aqueous solution of sodium citrate or the like is enclosed inside the above main body container 2.

It is preferred that the volume of the above main body container 2 is 20 to 80 ml. The reason for this is that if it is not larger than 20 ml, it is not possible to keep blood for testing at a sufficient amount, and if it is not less than 80 ml, blood is collected at a larger amount than required, so that excess blood is wasted.

Fig. 2 shows another example of the invention. In a container 11 for preserving, a blood collecting tool 3 is attached to the upper end of a main body container 2. The blood collecting tool 3 has a soft tube 6 for introducing blood, a pricking needle 7 attached to the end part of the tube 6, a connecting member 9 placed in the middle of the tube 6, a sampling port part 12 placed in the tube 6 on its end side than the connecting member 9 and a clamp 10 placed in the middle between the connecting member 9 and the sampling port part 12.

The pricking needle 7 is designed so that after a blood vessel of a blood donor is pricked with the pricking needle, a wing 7a is fixed to his arm or the like by means of an adhesive tape.

The connecting member 9 is provided with a soft tube 9a which upper and lower ends are connected to the above tube 6 for introducing blood, a large diameter piece 9c having a cylindrical shape made of hard resin inserted into the soft tube 9a, and a small diameter piece 9b molded integrally with said large diameter piece 9a closing the upper end part of the large diameter piece 9c. When the small diameter piece 9b is pressed from the exterior of the soft tube 9a with fingers or the like, a thin part 9d formed in the boundary part between the large diameter piece 9c and the small diameter piece 9d is broken and the upper end part of the large diameter piece 9c is opened, whereby a liquid flows through the large diameter piece 9c into the tube 6 for introducing blood.

The sampling port part 12 is provided in a large diameter tube 12b connected to the tube 6 for introducing blood and has a button 12a made of rubber for mixed injection. The structure of this button 12a made of rubber for mixed injection in the sampling port part 12 is the same as that of the above described sampling port part 8. The structure of the main body container 2 is the same as that of the container 1 illustrated in Fig. 1 so that its explanation is omitted.

Fig. 3 shows still another example of the invention. A main body container 21 for preserving has a main body container 2 to which the above described blood collecting tool 3 is connected, containers 4a and 4b for separating blood components and a container 4c for enclosing a diluting liquid. Instead of the sampling port part 8 in the above container 11 for preserving, a connecting member 5 is connected to the upper end part of the main body container 2, and a connecting tube 20 is

connected to the connecting member 5. A branching tube 21 is connected to the connecting tube 20, and connecting tubes 16 and 17 are connected to the branching tube 21. The end of the connecting tube 16 is connected to the upper end part of the container 4a for separating blood components, and the end part of the connecting tube 17 is connected through a connecting member 14 to the container 4b for separating blood components.

To the upper end part of the container 4a for separating blood components, a sampling port part 8 having a button 8a made or rubber for mixed injection is attached. Furthermore, the container 4b for separating blood components and the container 4c for enclosing diluting liquid are connected with each other through a connecting tube 18, and a sampling port part 13 is attached to the connecting tube 18 at the upper end part of the container 4b for separating blood components. A diluting liquid 31 such as a physiological salt aqueous solution, a phosphoric acid buffer solution or the like is enclosed in the container 4c for enclosing a diluting liquid.

The material for the containers 4a, 4b and 4c, the structure of the connecting member 5, 14 and 15, and the structure of the sampling port part 13 are the same as those explained in Fig. 1 or Fig. 2. The connecting tubes 16, 17, 18 and 20 are made of soft synthetic resin.

Examples of the application of the container 21 for preserving shown in Fig. 3 are explained. Firstly, after pricking a blood vessel of a blood donor with the prick needle 7, blood is introduced to the main body container 2 through the tube 6 for introducing blood and preserved. Before carrying out a blood test, this preserved blood in the main body container 2 by subjecting centrifuge separation of the container 21 for preserving segregate into blood components of a plasma layer/ a buffy coat layer/ an erythrocyte layer. Thereafter, the connecting member 5 of the main body container 2 is opened, and the main body container 2 is gradually pressed from the bottom to the top to thereby extrude the supernatant plasma layer and transfer it through the connecting tubes 20 and 16 to the container 4a for separating blood components. Then, after closing the connecting tube 16 with a clamp or the like, which is not shown in the figure, or cutting the connecting tube 16 by heat-sealing, the connecting means 14 of the container 4b for separating blood components is opened, and the buffy coat layer in the main body container 2 is transferred in the same manner as described above through the connecting tubes 20 and 17 to the container 4b for separating blood components. After finishing these operations, the erythrocyte layer remains in the main body container 2.

The blood components in the above main body containers 2, 4a and 4b are collected by pricking the sampling port parts 12, 8 and 13 with a blood collecting needle of a syringe or the like. The erythrocyte layer in the main body container 2 is used for test of erythrocytes, the plasma layer in the container 4a for separating is used for test of plasma, proteins, platelets and the like and the buffy coat layer in the container 4b for separating is used for test of lymphocytes. There are cases that the lymphocytes in the buffy coat layer do not have a sufficient amount of liquid. In such cases, the lymphocytes can be diluted by opening the connecting part 15 of the container 4c for enclosing a diluting liquid and introducing the diluting liquid 31 through the connecting tube 18 to the container 4b for separating.

Fig. 4 shows the relation between the days of preservation of preserved lymphocytes collected from the main body container 2 and the viability of the lymphocytes. From this figure, it is understood that the activity of the preserved lymphocytes is kept at not less than 95% the initial value even at 13th day of preservation.

Next, lymphocytes of blood-donors A and B collected and preserved using the above container 21 for preserving were mixedly cultured to examine the reactivity of T lymphocytes (responder). Firstly, the lymphocytes of the blood donor A or B were treated as stimulating cells (Stimulater) by irradiating them with X ray (or MMC) to eliminate their ability of propagation. The above lymphocytes were mixed with those to react therewith, that is, T lymphocytes of the donor A or B, and the reactivity of T lymphocytes was examined by stimulating and measuring the degree of cellular propagation based on the incorporation of 3H-thymidine.

Fig. 5 shows the results of the examination, wherein black dots indicate the stimulation index between the same blood donor A-A or B-B, and an white circles indicate the stimulation index between different blood donors A-B. In this figure, the stimulation indexes of the preserved lymphocytes show a ratio that permits to judge the culture of mixed lymphocytes even after 3 days of preservation. Actually, the culture of mixed lymphocytes could be judged after 3 days of preservation. Moreover, judgement of class 1 antigen and class 2 antigen could be achieved satisfactorily even after 7 days of preservation.

The stimulation indexes (SI) were expressed as a reaction between blood donors A-B divided by a reaction between a blood donor A-A or B-B. When SI is not larger than 2.0. MLC is judged as (-), that is, judged to be compatible. When SI is not less than 5.0, MLC is judged as (+), that is, judged to be not compatible. Fig. 5 shows MLC of the those which were clearly not compatible with each other, and the result that MLC was (+) was obtained at

least for 3 days of preservation.

According to the container of the invention for preserving blood for testing, the collecting of blood, the preservation of blood and the component separation of preserved blood can be carried out in a closed circuit, so that the samples can be kept asepsis. In addition to this effect, the container has proper oxygen permeability, so that the blood for testing can be preserved at least for not less than 3 days at room temperature. Furthermore, according to the invention, the measurement of the ratio of surface markers of lymphocytes, the judgement of the compliment and the like are possible even after 3 days after blood is collected. Moreover, since the container according to the invention is constructed using soft plastics, such problems as suffering from damage during transportation can be avoided.

## Claims

1. A container which preserves blood to be tested for a long time at room temperature,

   provided with a bag-like main body container made of a flexible synthetic resin and a sampling port part incorporated in the main body container for collecting blood components,

   wherein the above main body container has a volume of 20 to 80 ml, in which an anti-coagulant is enclosed in advance, and the flexible synthetic resin has oxygen permeability of not less than 0.1 ml/ 24 hr/atm per 1 ml of whole blood.

2. The container for preserving blood for testing as claimed in claim 1,

   wherein a blood collecting means is connected to the main body container, and the blood collecting means is provided with

   a tube for introducing blood to the inside of the container;

   a needle for collecting blood attached to the end of said tube for introducing blood;

   a connecting member provided in the middle of said tube for introducing blood, having a closing member for closing the flow path of said tube for introducing blood before use and having a structure that the flow path is opened by breaking the closing member when used; and

   a clamp for closing the above tube for introducing blood, and the sampling port part is provided in the above tube for collecting blood.

3. The container for preserving blood for testing as claimed in claim 2,

   wherein a container for preserving blood for receiving separated blood components is connected through a connecting tube to the main body container, and the container for preserving blood is provided with a sampling port part for collecting blood components in said container for preserving blood.

4. The container for preserving blood for testing as claimed in claim 2,

   wherein a first container for receiving blood components and a second container for receiving blood components are connected through a first connecting tube to the main body container, and a container in which a diluting liquid is enclosed is connected through a second connecting tube to the second container for receiving blood components,

   the above first connecting tube connecting the above main body container and the first container for receiving blood components through a connecting member,

   the above second connecting tube connecting through a connecting member to the above container in which a diluting liquid is enclosed, and

   each of the above first and second containers for receiving blood components being provided with a sampling port part for collecting blood components therein.

5. A container for preserving blood for testing having a volume of 20 to 80 ml made of a flexible synthetic resin having oxygen permeability of not less than 0.1 ml/ 24 hr/atm per 1 ml of whole blood in which an anti-coagulant is enclosed in advance, and when blood lymphocytes received with asepsis in said container is preserved at room temperature for at least not less than 3 days, not less than 90% of the lymphocyte activity is retained.

FIG_1

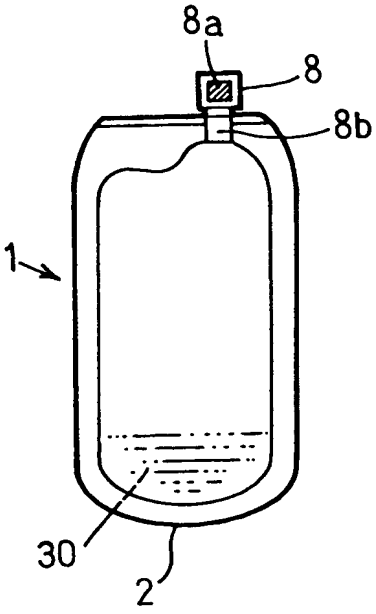



FIG_2

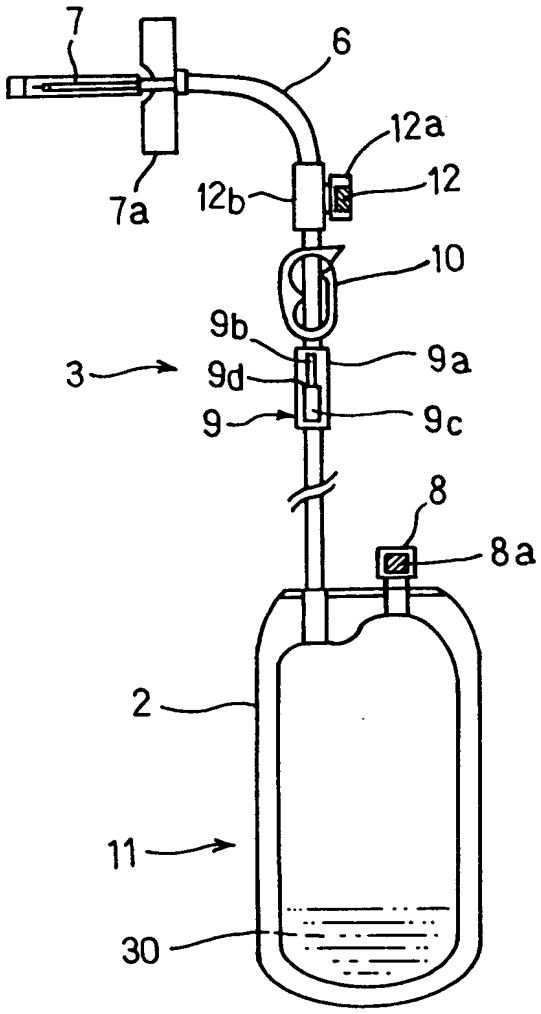


FIG_3

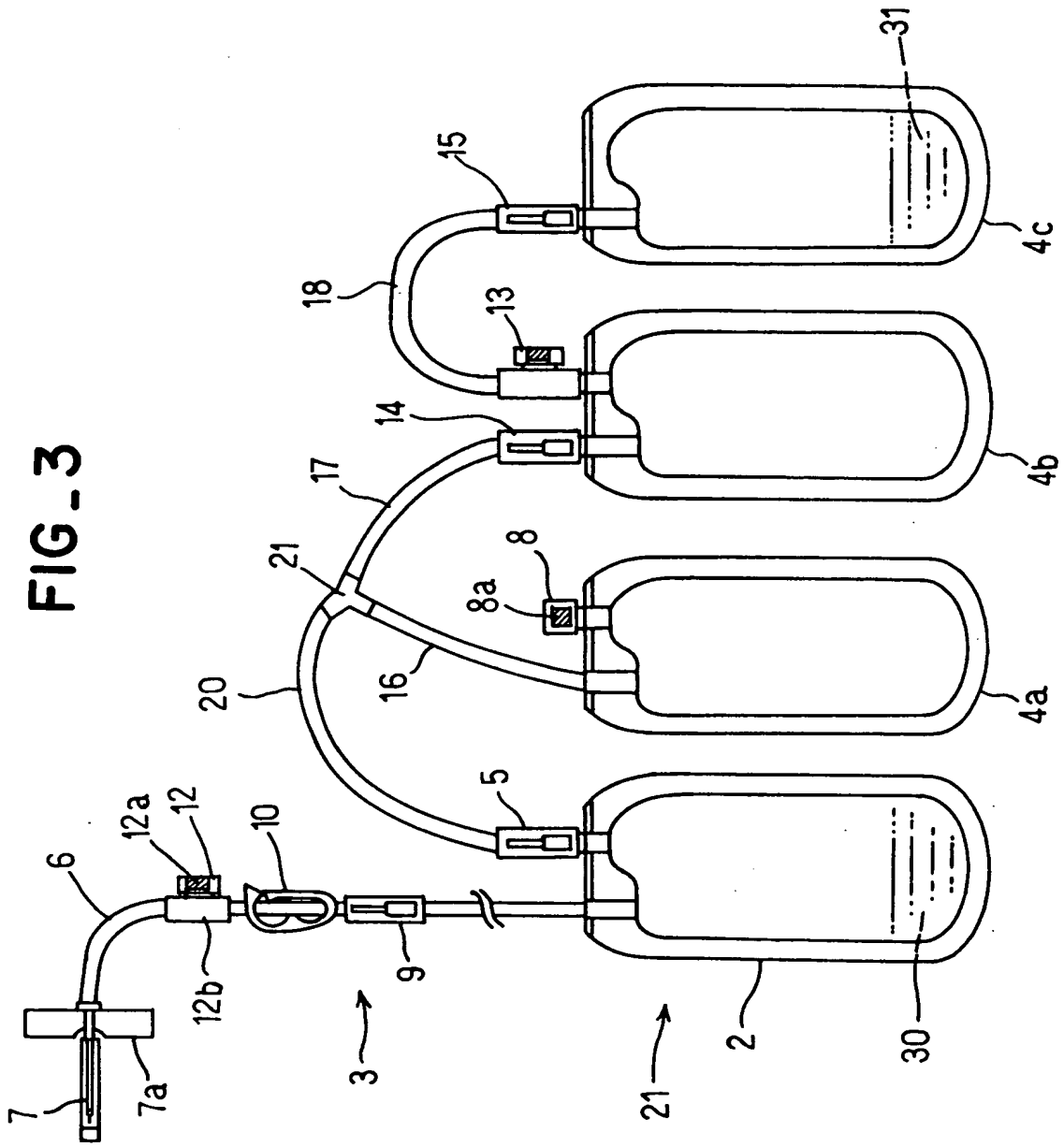


FIG_4

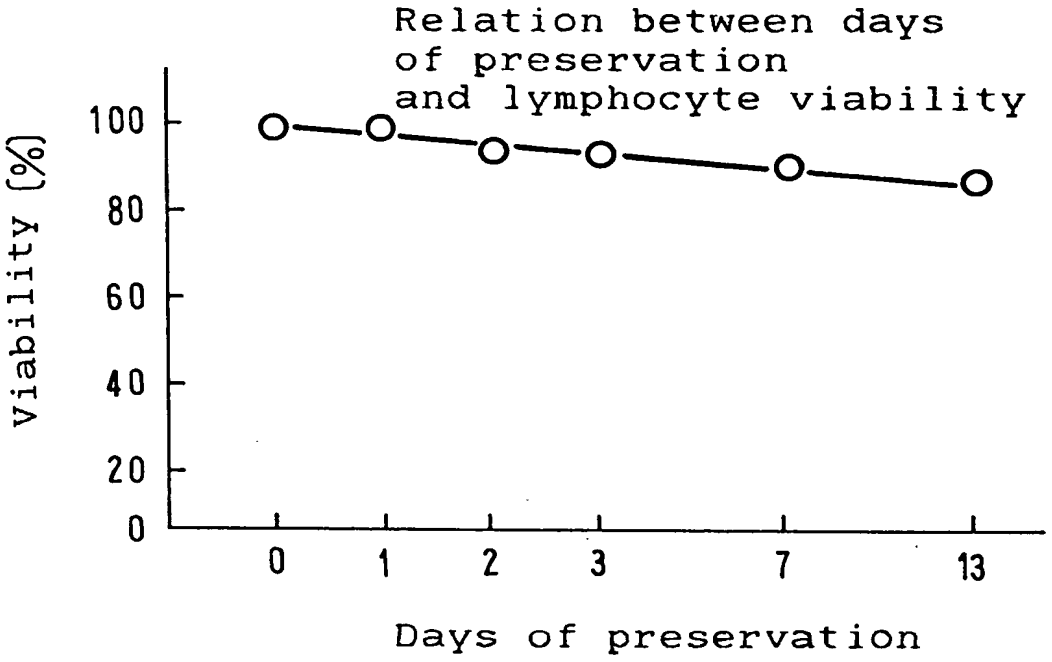
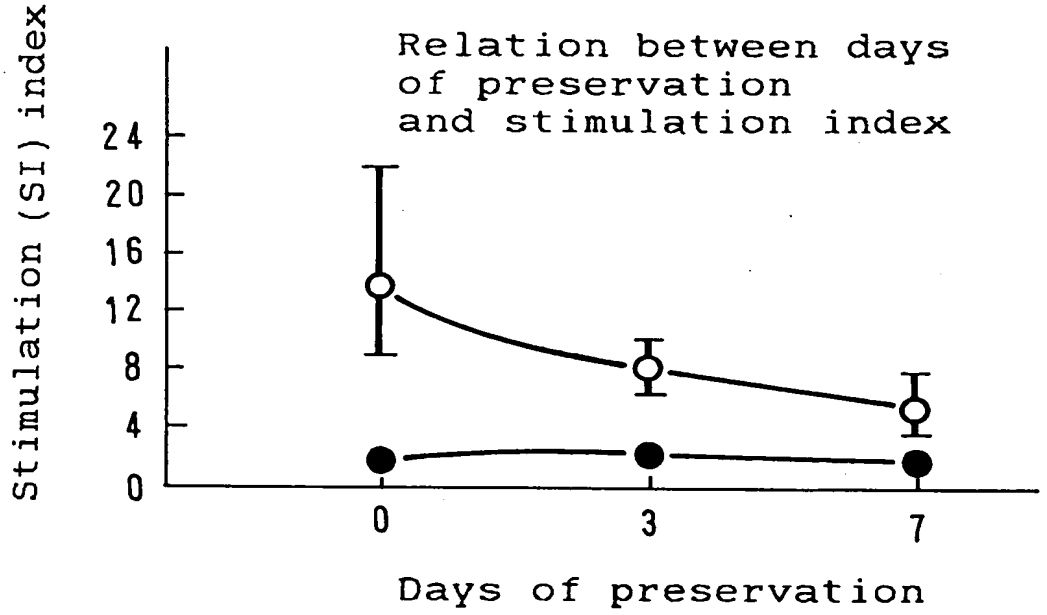

Relation between days
of preservation
and lymphocyte viability
Viability (%)
100
80
60
40
20
0
0
1
2
3
7
13
Days of preservation

FIG_5


Relation between days
of preservation
and stimulation index
Stimulation (SI) index
24
20
16
12
8
4
0
0
3
7
Days of preservation

European Patent Office

# EUROPEAN SEARCH REPORT

Application Number

EP 91 11 2710

## DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int. Cl.5) |
|---|---|---|---|
| Y | EP-A-0 074 178 (E.I. DUPONT DE NEMOURS)<br>* page 4, lines 9-20; page 5, line 7 - page 7, line 4 * | 1-5 | A 61 J 1/00 |
| Y | EP-A-0 054 221 (CUTTER LABORATORIES)<br>* page 3, line 6 - page 7, line 23; figure 1 * | 1-5 | |
| A | EP-A-0 114 964 (MILES LABORATORIES)<br>* page 11, lines 15-34; page 20, line 20 - page 23, line 24; figure 3 * | 1,2,5 | |
| A | EP-A-0 186 018 (BIOTEST)<br>* page 5, line 14 - page 6, line 26; figure 1 * | 1,5 | |

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

A 61 J
A 61 M

The present search report has been drawn up for all claims

| Place of search | Date of completion of search | Examiner |
|---|---|---|
| Berlin | 07 November 91 | MONNE E.M.B. |

CATEGORY OF CITED DOCUMENTS

X: particularly relevant if taken alone
Y: particularly relevant if combined with another document of the same catagory
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